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A new cave species of the genus *Triplophysa* (Teleostei, Cypriniformes, Nemacheilidae) from Guizhou, China

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Abstract

Integrating morphological and molecular evidence, we described a new species of *Triplophysa*, named *Triplophysa xingyiensis* **sp. nov.**, from two subterranean river outlets in the Nanpanjiang River Basin, Guizhou Province, China. This new species can be distinguished from other species of *Triplophysa* by the following combination of characters: body naked and scaleless, eyes normal with diameter 9.1–17.3% of head length; lateral line complete; posterior chamber of air bladder degenerated; out rostral barbel longest, length 34.3–48.0% of head length; caudal fin emarginated, with 16 branched fin rays; and vertebrae 4 + 36. Phylogenetic analysis, based on the 13 protein-coding genes of the mitochondrial genome, demonstrated that the new species forms a monophyletic group. Furthermore, analysis of genetic distances, based on the cytochrome *b* gene, revealed its close relationship with *T. anlongensis*, *T. rongduensis* and *T. zhenfengensis*, with pairwise distances ranging from 1.8% to 2.4%. This integrative taxonomic method supports the recognition of *T. xingyiensis* as a distinct taxon.

Key Words

Cavefish, molecular analysis, morphology, Nanpanjiang River

Introduction

The genus *Triplophysa* Rendahl, 1933 belongs to the order Cypriniformes and the family Nemacheilidae (Zhu 1989). This genus comprises numerous species with distributions confined to streams, rivers and lakes across the Qinghai-Tibet Plateau (QTP) and its adjacent regions, with over 140 species having been documented worldwide (Zhu 1989; Prokofiev 2010; Kottelat 2012; Fricke et al. 2025). *Triplophysa* can be distinguished from other genera in the Nemacheilidae family by the following unique morphological characteristics: scales reduced or absent, nostrils positioned closer together and the posterior wall of the lateral swim bladder capsule being ossified (Zhu 1989; Prokofiev 2010; Ren et al. 2012; Chen and Peng 2019). Based on habitat preferences, *Triplophysa* species are classified as either epigean (surface–dwelling) or hypogean (cave–dwelling) (Liu et al. 2017; Lu et al. 2022). Hypogean species, in particular, exhibit specialised adaptations to subterranean environments, including reduced or absent eyes, scaleless bodies, loss or reduction of pigmentation and elongated pectoral and pelvic fins (Borowsky 2018; Luo et al. 2023). These adaptations reflect their evolutionary response to the unique challenges of cave ecosystems, thereby enabling the fish to efficiently forage and navigate their lightless surroundings. As of now, multiple studies have given accounts of 44 species of *Triplophysa* cave loaches that inhabit the karst region in southwest China (Lan et al. 2024; Cao et al. 2025; Shi et al. 2025; Zhu et al. 2025).

Guizhou Province is located in the south-western part of China, bordered by Guangxi, Hunan, Yunnan, Sichuan and Chongqing (Department of Natural Resources of Guizhou

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Province 2024). Famous for its classic karst landscape, the Province features many underground rivers and caves, making it a priority area for biodiversity conservation (Liu et al. 2018). It is regarded as a biodiversity hotspot area for cave fish in China and a biodiversity conservation area that requires priority protection (Zheng et al. 2009; Wang et al. 2019). Currently, there are 15 recorded species of the *Triplophysa* genus of cave fish in this region. These include *T. nasobarbatula*, *T. zhenfengensis*, *T. longliensis*, *T. baotianensis*, *T. guizhouensis*, *T. sanduensis*, *T. qingzhenensis*, *T. wudangensis*, *T. cehengensis*, *T. rongduensis*, *T. panzhouensis*, *T. anlongensis*, *T. ziyunensis*, *T. yaluwang* and *T. xiuwenensis* (Wang and Li 2001; Ren et al. 2012; Li et al. 2018; Wu et al. 2018; Chen and Peng 2019; Liu et al. 2022; Luo et al. 2023; Lan et al. 2024; Zhu et al. 2025).

During an investigation in July 2024 and July 2025, several specimens of the genus *Triplophysa* were collected from two subterranean river outlets in Xingyi City, Guizhou Province, with their distribution sites being the same as those of *Sinocyclocheilus xingyiensis* (Luo et al. 2024). Morphological comparisons and molecular phylogenetic analyses showed that these specimens were identified as the same species and belonged to a new species within the genus *Triplophysa*.

Materials and methods

Specimen sampling and preservation

All *Triplophysa* specimens were collected with hand nets from two subterranean river outlets in Xingyi City, Guizhou Province, China (Fig. 1). After collection, the fish were euthanised in compliance with the relevant laws of the Chinese Laboratory of Animal Welfare and Ethics (GB/T 35892-2018). All specimens were fixed in 10% buffered formalin and subsequently transferred to 75% ethanol for long-term preservation. Muscle tissue samples for molecular analysis were preserved in 75% ethanol and stored under low-temperature conditions. All voucher specimens are deposited in the School of Life Sciences, Guizhou Normal University (GZNUSLS), Guiyang, Guizhou Province, China.

Morphological comparisons

We used digital calipers to measure the measurable traits of nine straight-bodied, fully intact specimens of the new species *Triplophysa*. All measurements were recorded to the nearest 0.1 mm. All counts and measurements follow the methods of Ren et al. (2012) and Tang et al. (2012). Moreover, we collected and examined samples of 25 species of *Triplophysa* from the type localities, encompassing: *T. anlongensis*, *T. baotianensis*, *T. cehengensis*, *T. erythraea*, *T. guizhouensis*, *T. huapingensis*, *T. nasobarbatula*, *T. langpingensis*, *T. longliensis*, *T. nanpanjiangensis*, *T. panzhouensis*, *T. qingzhenensis*, *T. qiubeiensis*, *T. rongduensis*, *T. rosa*, *T. sanduensis*, *T. tianxingiensis*, *T. tianeensis*, *T. wudangensis*, *T. xiangshuingensis*, *T. xiuwenensis*, *T. yangi*, *T. yunnanensis*, *T. zhenfengensis* and *T. ziyunensis* (see Appendix 1). Furthermore, a specimen of the new species was X-rayed using a Micro Computed Tomography

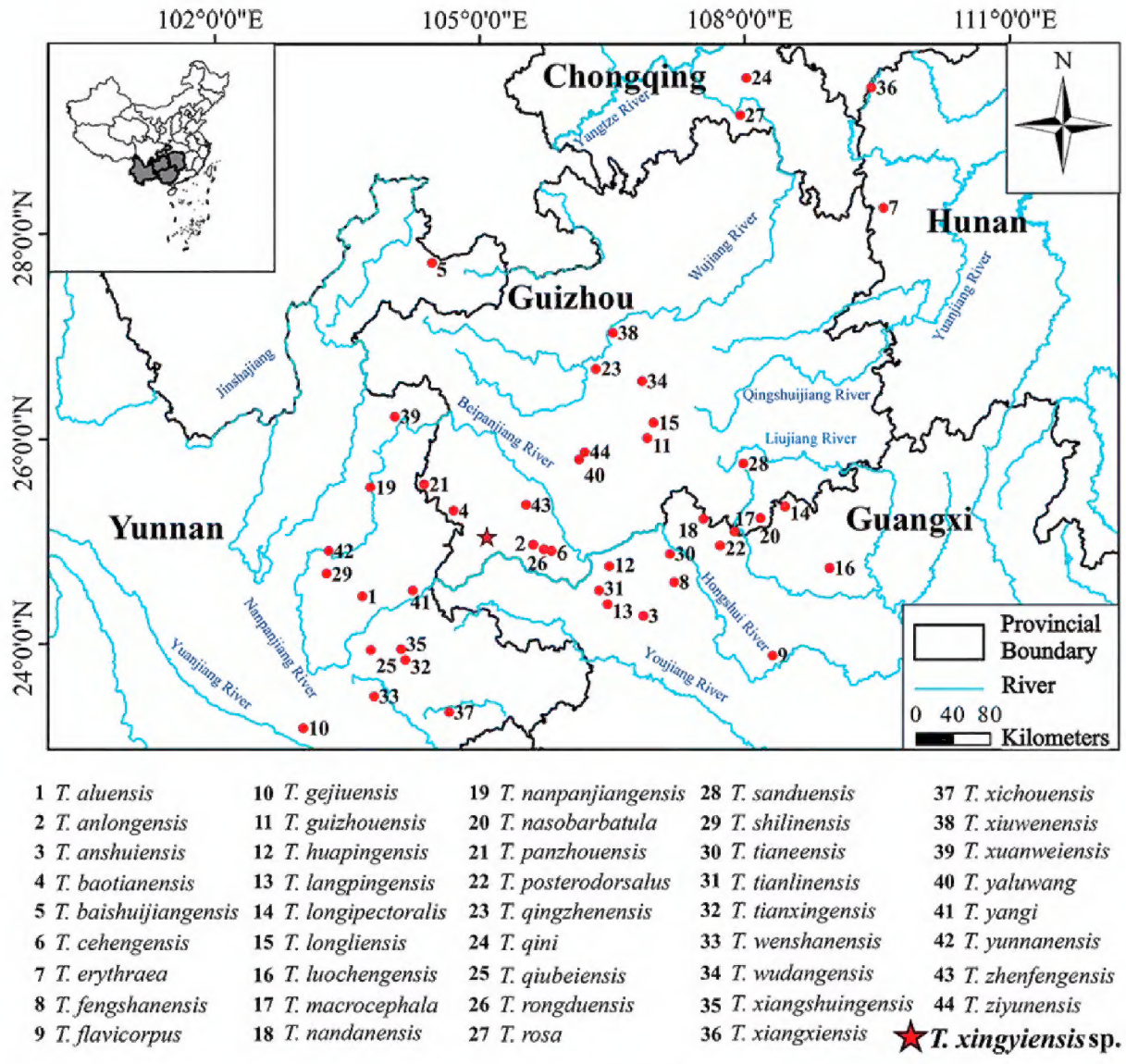

Figure 1. Sampling locality of *Triplophysa xingyiensis* sp. nov. and 44 hypogean species of the genus *Triplophysa* in southern China. The base maps originate from the Standard Map Service website (http://bzdt.ch.mnr.gov.cn/).

(Bruker Skyscan 1276) to count the vertebrae. Abbreviations used in this study include TL for total length, SL for standard length and HL for head length. Morphometric and meristic data were tabulated and analysed using Microsoft Excel.

DNA extraction, amplification and sequencing

In this study, genomic DNA was first extracted from samples using a universal genomic DNA extraction kit (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China). The integrity, purity and concentration of the extracted DNA were then rigorously assessed via agarose gel electrophoresis, NanoDrop2000 and Quantus Fluorometer (Picogreen), respectively. Qualified DNA samples were fragmented using Covaris M220 (Gene Company, China) and fragments of approximately 400 bp were selected for the construction of paired-end (PE) libraries. Library preparation for each sample was performed using the NEXTFLEX Rapid DNA-Seq Kit. The constructed libraries were sequenced on the DNBSEQ-T7 platform (MGI, Shenzhen, China) in PE150 mode by Majorbio Bio-Pharm Technology Co., Ltd. Finally, based on the raw sequencing data, complete animal mitochondrial genomes were assembled using GetOrganelle (Jin et al. 2020) and the cytochrome *b* (Cyt *b*) gene sequences were extracted from the mitochondrial genomes for genetic distance calculation.

Phylogenetic analyses

This study constructed a dataset of 68 samples, based on the 13 protein-coding genes from the mitochondrial genome. The sequences were sourced from 54 public entries in the NCBI GenBank database and 14 samples that were collected and sequenced by our team (including mitochondrial genomes from two individuals of a new species) (Table 1). Multiple sequence alignment was performed using MAFFT v.7.505 (Katoh and Standley 2013), from which informative sites were selected for phylogenetic analysis and the filtered sequences were subsequently employed for phylogenetic reconstruction. Bayesian Inference analysis was conducted using MrBayes v.3.2.6 (Ronquist et al. 2012) integrated within PhyloSuite v.1.2.3 (Zhang et al. 2020; Xiang et al. 2023). Four Markov Chain Monte Carlo (MCMC) chains were run simultaneously for 2 million generations, sampling every 1000 generations. After discarding the initial 25% of samples as burn-in, a majority-rule consensus tree was generated from the remaining samples and Bayesian Posterior Probabilities (BPP) were calculated. Based on the Cyt *b* sequences, pairwise genetic distances were calculated using the Kimura-2-Parameter (K2P) model (Kimura 1980) in MEGA v.11.0 (Tamura et al. 2021). Phylogenetic trees were visualised using the Interactive Tree Of Life (iTOL; https://itol.embl.de/).

Results

***Triplophysa xingyiensis* Yuan, Zhu & Zhang, sp. nov.**

https://zoobank.org/9DCEF788-B111-4930-90B8-13AE38760653

Figs 2–4, Table 2

Type materials. ***Holotype.*** GZNUSLS202507016, 38.3 mm SL, was collected by Ren-Yi Zhang, Feng-Hua Yuan, Ting-Ting Zhu and Ping Yu in July 2025, at Zhengtun Town, Xingyi City, Guizhou Province, China (25.0488°N, 105.0222°E; ca. 1241 m Alt.; Fig. 2).

Paratypes. GZNUSLS202407018, 35.1 mm SL, collected from the same locality as the holotype; GZNUSLS202407013, 46.3 mm SL; GZNUSLS202407024, 59.2 mm SL; GZNUSLS202507023–GZNUSLS202507027, 38.20–77.5 mm SL, collected from the Dingxiao subdistrict, Xingyi City, Guizhou Province, China (25.1570°N, 105.0014°E; ca. 1272 m Alt.). Collected by Ren-Yi Zhang, Ting-Ting Zhu, Lei-Shan Wang, Feng-Hua Yuan, and Ping Yu in July 2024 and 2025.

Diagnosis. *Triplophysa xingyiensis* sp. nov. can be distinguished from other species of *Triplophysa* by the following combination of characters: (1) Eyes normal, eye diameter 9.1–17.3% of head length (HL); (2) interorbital width 29.3–37.5% of HL; (3) anterior and posterior nostrils closely set, anterior nostril elongated into a barbel-like tip; (4) outer rostral barbel extending posteriorly to the anterior margin of the eye; (5) body naked and scaleless, with skin pigmentation; (6) lateral line complete; (7) dorsal fin distal margin truncated; (8) pectoral fin tip not reaching the origin of the pelvic fin; (9) pelvic fin tip not reaching the anus; (10) dorsal fin rays ii, 7; pectoral fin rays ii, 7–9; pelvic fin rays ii, 5; anal fin rays i, 5; 16 branched caudal fin rays; (11) Total vertebrae 4 + 36 (Table 2, Fig. 3).

Description. The morphological measurements were conducted on *Triplophysa xingyiensis* sp. nov. (Table 2). The body is smooth and scaleless, with skin pigmentation, elongated and cylindrical, gradually compressed from the dorsal to the caudal fin base. The maximum body depth anterior to the dorsal fin origin was 13.5–15.2% of standard length. The dorsal profile was slightly convex from the snout to the dorsal fin origin and straight from the posterior dorsal fin to the caudal fin base. The ventral profile was flat.

The head is moderately long, 20.1–24.3% of standard length, slightly compressed and flattened with width exceeding depth (head width/head depth = 1.0–1.3). The snout is short, 40.2–54.6% of head length. The mouth is inferior and curved, with corners positioned below the anterior nostrils. The lips are smooth, thin and shallowly wrinkled; the lower lip has a V-shaped median incision. No dental processes were present. Three pairs of barbels were present: inner rostral barbels were short (19.3–30.2% of head length), extending posteriorly to the corners of the mouth; outer rostral barbels were longer (34.3–48.0% of head length), reaching the mid-point of the eye; and maxillary barbels were shorter (30.1–44.4% of head length), with tips not reaching the anterior margin of the operculum. The anterior and posterior nostrils are closely spaced, with the anterior nostril forming a long tube terminating

Table 1. A list of 68 species and sequence information used for molecular analysis.

ID	Species	Localities	Drainage	Genbank accession numbers	
				Cyt *b*	Mitogenome
1	*T. alticeps*	Qinghai, China	Qinghai Lake	/	KT213585
2	*T. aluensis*	Yunnan, China	Nanpanjiang River	/	/
3	*T. angeli*	Sichuan, China	Yalongjiang River	/	MZ325251
4	*T. anlongensis*	Guizhou, China	Nanpanjiang River	PP204904	PP204904
5	*T. anshuiensis*	Guangxi, China	Hongshui River	/	/
6	*T. anterodorsalis*	Sichuan, China	Yellow River	/	KJ739868
7	*T. baishuijiangensis*	Yunnan, China	Jinsha River	PQ199313	/
8	*T. baotianensis*	Guizhou, China	Nanpanjiang River	MT992550	MT992550
9	*T. bleekeri*	Chongqing, China	Yellow River	/	JQ686729
10	*T. brevicauda*	Sichuan, China	Yangtze River	/	KT213588
11	*T. cehengensis*	Guizhou, China	Beipanjiang River	PP204902	PP204902
12	*T. dorsalis*	Xinjiang, China	Irtysh River	/	KT213591
13	*T. erythraea*	Hunan, China	Yuanjiang River	PP455386	PP455386
14	*T. fengshanensis*	Guangxi, China	Hongshui River	OQ998929	OQ998929
15	*T. flavicorpus*	Guangxi, China	Hongshui River	/	/
16	*T. gejiuensis*	Yunnan, China	Nanpanjiang River	/	/
17	*T. guizhouensis*	Guizhou, China	Hongshui River	this study	this study
18	*T. huapingensis*	Guangxi, China	Hongshui River	PP204899	PP204899
19	*T. langpingensis*	Guangxi, China	Hongshui River	this study	this study
20	*T. lixianensis*	Sichuan, China	Yellow River	/	KT966735
21	*T. longipectoralis*	Guangxi, China	Liujiang River	OQ998928	OQ998928
22	*T. longliensis*	Guizhou, China	Hongshui River	OQ998931	OQ998931
23	*T. luochengensis*	Guangxi, China	Hongshui River	/	/
24	*T. macrocephala*	Guangxi, China	Liujiang River	PP204900	PP204900
25	*T. markehenensis*	Qinghai, China	Minjiang River	/	KT213594
26	*T. nandanensis*	Guangxi, China	Hongshui River	OQ998932	OQ998932
27	*T. nasobarbatula*	Guizhou, China	Liujiang River	ON116529	ON116529
28	*T. nanpanjiangensis*	Yunnan, China	Nanpanjiang River	this study	this study
29	*T. pappenheimi*	Gansu, China	Yellow River	/	KT213600
30	*T. panzhouensis*	Guizhou, China	Nanpanjiang River	this study	this study
31	*T. posterodorsalus*	Guangxi, China	Liujiang River	/	/
32	*T. qingzhenensis*	Guizhou, China	Wujiang River	this study	this study
33	*T. qini*	Chongqing, China	Yuanjiang River	ON528184	/
34	*T. qiubei*	Yunnan, China	Nanpanjiang River	this study	this study
35	*T. robusta*	Gansu, China	Yellow River	/	KM396312
36	*T. rongduensis*	Guizhou, China	Nanpanjiang River	PP204903	PP204903
37	*T. rosa*	Chongqing, China	Wujiang River	JF268621	JF268621
38	*T. sanduensis*	Guizhou, China	Duliujiang River	this study	this study
39	*T. shilinensis*	Yunnan, China	Nanpangjiang River	/	/
40	*T. siluroides*	Gansu, China	Yellow River	/	KT213603
41	*T. stenura*	Yunnan, China	Nujiang River	/	KX354975
42	*T. stewarti*	Tibet, China	Lhasa River	/	KJ631324
43	*T. stoliczkai*	Qinghai, China	Yellow River	/	JQ663847
44	*T. strauchii*	Xinjiang, China	Irtysh River	/	KP297875
45	*T. tianeensis*	Guangxi, China	Hongshui River	PP182257	PP182257
46	*T. tianlinensis*	Guangxi, China	Hongshui River	/	/
47	*T. tianxingensis*	Yunnan, China	Nanpangjiang River	PV394926	/
48	*T. tibetana*	Xizang, China	Brahmaputra River	/	KT122845
49	*T. venusta*	Yunnan, China	Yangtze River	/	KT008666
50	*T. weiheensis*	Gansu, China	Yellow River	/	PP203140
51	*T. wenshanensis*	Yunnan, China	Panlonghe River	PP661512	/
52	*T. wudangensis*	Guizhou, China	Wujiang River	PP204906	PP204906
53	*T. xiangshuingensis*	Yunnan, China	Nanpanjiang River	this study	this study
54	*T. xiangxiensis*	Hunan, China	Yuanjiang River	KT751089	KT751089
55	*T. xichouensis*	Yunnan, China	Red River	/	/
56	*T. xiuwenensis*	Guizhou, China	Wujiang River	this study	this study
57	*T. xuanweiensis*	Yunnan, China	Beipanjiang River	OL675198	/
58	*T. yaluwang*	Guizhou, China	Hongshui River	PQ117067	/
59	*T. yangi*	Yunnan, China	Nanpanjiang River	PQ356185	PQ356185

ID	Species	Localities	Drainage	Genbank accession numbers	
				Cyt *b*	Mitogenome
60	*T. yunnanensis*	Yunnan, China	Nanpanjiang River	this study	this study
61	*T. zhenfengensis*	Guizhou, China	Beipanjiang River	this study	this study
62	*T. ziyunensis*	Guizhou, China	Hongshui River	this study	this study
63	*T. xingyiensis* **sp. nov.**	Guizhou, China	Nanpanjiang River	this study	this study
64	*T. xingyiensis* **sp. nov.**	Guizhou, China	Nanpanjiang River	this study	this study
65	*Claea wulongensis*	Chongqing, China	Yangtze River	/	PP025975
66	*Barbatula barbatula*	/	/	/	KP715096
67	*Homatula berezowskii*	Yunnan, China	Yangtze River	/	NC_040302
68	*H. potanini*	Yunnan, China	Yangtze River	/	NC_025321

Table 2. Main morphometric characters of *Triplophysa xingyiensis* sp. nov.

Item	Holotype	Holotype + Paratypes (n = 9)		
		Range	Mean	SD
Total length (TL, mm)	47.7	43.4–90.2	63.8	19.2
Standard length (SL, mm)	38.3	35.1–77.5	52.9	17.0
Head length (HL, mm)	9.1	7.7–18.6	12.1	4.2
In percent SL (%)				
Head length	23.9	20.1–24.3	22.9	1.5
Body depth	15.2	13.5–15.2	14.4	0.7
Body width	10.7	9.3–14.8	12.1	1.7
Pre–dorsal length	48.3	48.3–52.5	50.1	1.5
Pre–pectoral length	26.5	22.6–26.5	24.8	1.4
Pre–pelvic length	49.5	49.4–53.1	51.0	1.3
Pre–anal length	75.9	72.7–80.2	75.8	2.3
Pre–anus length	67.8	67.8–73.9	70.3	1.9
Dorsal fin length	20.7	16.0–20.7	18.8	1.7
Pectoral fin length	19.5	15.2–19.8	17.8	1.7
Pelvic fin length	17.6	13.5–17.6	15.1	1.5
Anal fin length	17.7	13.6–17.7	15.1	1.3
Caudal peduncle length	17.2	15.6–19.2	17.1	1.3
Caudal peduncle depth	9.7	7.2–10.1	9.2	0.9
Dorsal fin base length	12.0	8.7–12.0	10.7	1.2
Pectoral fin base length	4.2	3.4–5.1	4.1	0.6
Pelvic fin base length	3.3	2.8–5.4	3.6	0.8
Anal fin base length	7.5	6.0–7.5	6.8	0.4
In percent HL (%)				
Head depth	46.8	46.8–62.3	52.5	4.8
Head width	54.5	54.4–71.4	61.0	5.7
Snout length	40.2	40.2–54.6	46.3	4.6
Eye diameter	9.1	9.1–17.3	12.0	2.7
Interorbital width	37.5	29.3–37.5	33.7	2.7
Inter nostril width	21.8	17.5–29.0	21.5	3.3
Inner rostral barbel length	26.1	19.3–30.2	25.6	3.3
Maxillary barbel length	36.8	30.1–44.4	36.0	4.0
Outer rostral barbel length	42.0	34.3–48.0	42.3	4.5

in a short barbel-like tip that does not extend beyond the anterior margin of the eye. Eyes are present and normal, with a diameter of 9.1–17.3% of head length.

Dorsal–fin ii, 7; pectoral–fin ii, 7–9; pelvic–fin ii, 5; anal–fin i, 5; caudal–fin 16. The dorsal fin is moderately long (16.0–20.7% of SL) with a truncated distal margin. Its origin was slightly anterior to the pelvic–fin origin and positioned posterior to the mid-point between the snout tip and caudal fin base. The first branched ray is the longest, shorter than the head length. The dorsal fin tip does not reach the vertical through the anus. The pectoral fin is well developed (15.2–19.8% of SL), extending posteriorly to the mid-point between the pectoral and pelvic fin origins, but not reaching the pelvic fin origin. The pelvic fin length is 13.5–17.6% of SL; its tip does not reach the anus, with the distance between the pelvic–fin tip and anus being 0.3–3.2 times the eye diameter. The anal fin is short (13.6–17.7% of SL), with a truncated distal margin. Its origin was close to the anus (distance 1.2–4.4 mm). The anal fin tip did not reach the caudal fin base, with the distance between the anal–fin tip and anus being 5.3–11.9 times the eye diameter. The caudal fin is forked, with equally long, pointed lobes. The caudal peduncle length was 6.0–13.7 mm and its depth was 3.5–7.6 mm. No adipose keels were present on either side of the caudal peduncle. Total vertebrae: 4 + 36.

Cephalic lateral line canals are distinct; the lateral line is complete and straight, extending beyond the pectoral–fin tip to the caudal–fin base (Fig. 4). The posterior chamber of the bladder is degenerated, with the bony capsule forming a dumbbell shape.

Colouration. In the living environment, the body is light yellow, with irregular dark brown patches scattered on the head and dorsal portions of the lateral sides and small black blotches are present on the rays of the dorsal, pectoral and caudal fins (Fig. 2). After fixation in 10% formalin, the base of the pectoral fin is light yellow, the belly is white and the rest of the body is light grey (Fig. 4).

Distribution and habitat. This species is currently known only from karst subterranean rivers in Xingyi, Guizhou, China, which belong to the Nanpanjiang River system. Specimens of the new species were collected from a well, connected to the subterranean river. The cave entrance is no more than 1 metre wide and during the rainy season, water can be observed flowing out from the entrance (Fig. 5).

Sexual dimorphism. No secondary sex characteristics were observed on the specimens of *Triplophysa xingyiensis* sp. nov.

Etymology. Xingyi City, the sampling site, is the source of the species name. The Chinese common name is proposed as '兴义高原鳅'.

Comparisons. Presently, there are a total of 44 valid cave *Triplophysa* species recorded in southwest China.

Among them, *T. aluensis*, *T. gejiuensis*, *T. xiangxiensis*, *T. shilinensis*, *T. tianeensis*, *T. rosa*, *T. posterodorsalus*, *T. qiubeiensis*, *T. huapingensis*, *T. macrocephala*, *T. fengshanensis*, *T. langpingensis*, *T. xichouensis*, *T. luochengensis*, *T. tianlinensis*, *T. baotianensis*, *T. anshuiensis*, *T. erythraea*, *T. qingzhenensis*, *T. qini*, *T. wudangensis*, *T. xuanweiensis*,

Figure 2. Living individual of *Triplophysa xingyiensis* sp. nov., GZNUSLS202507016, 38.3 mm SL.

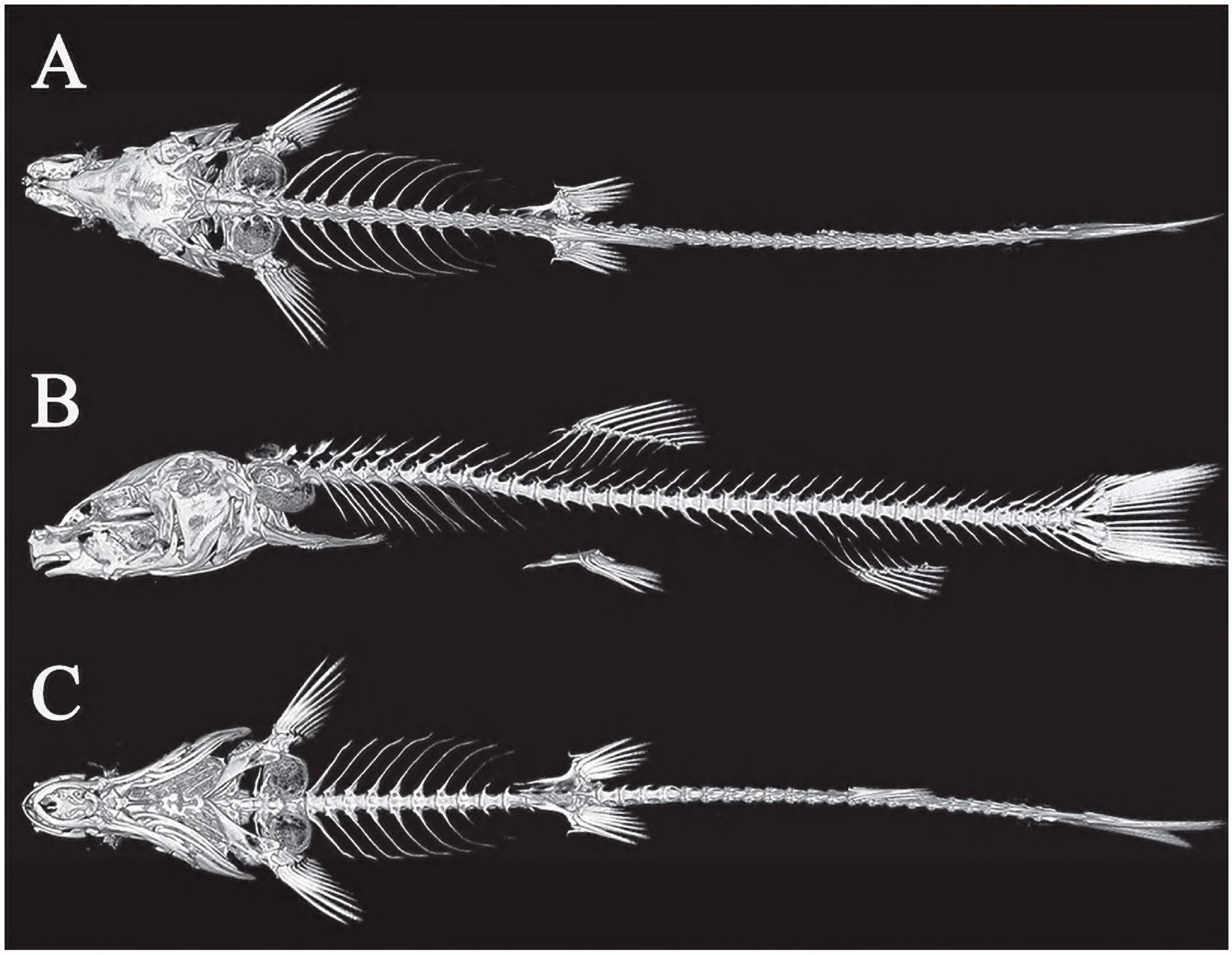

Figure 3. Micro–CT graph of *Triplophysa xingyiensis* sp. nov., GZNUSLS202407013, 46.3 mm SL. **A.** Dorsal view; **B.** Lateral view; **C.** Ventral view.

T. cehengensis, *T. yaluwang*, *T. ziyunensis*, *T. yangi*, *T. wenshanensis*, *T. xiuwenensis* and *T.baishuijiangensis* either lack eyes or have reduced eye structures. Therefore, *T. xingyiensis* sp. nov. can be easily distinguished from the above species by its normal eyes.

All *Triplophysa* species with normal eyes, including *T. xingyiensis* sp. nov., possess a complete lateral line and pigmentation on the body surface. However, *T. xingyiensis* sp. nov. differs from *T. flavicorpus*, *T. guizhouensis*, *T. longipectoralis*, *T. nasobarbatula*, *T. sanduensis* and *T. yunnanensis* by having no scales on its body surface (vs. having small scales on the body surface). It differs from *T. panzhouensis* by having distinct lateral head canals (vs. indistinct lateral head canals). *T. xingyiensis* sp. nov. differs from *T. longliensis* and *T. nandanensis* by the tip of the pelvic fin not reaching the anus (vs. the tip of the pelvic fin reaching the anus). It differs from *T. nanpanjiangensis* and *T. tianxingensis* by having a forked caudal fin (vs. the

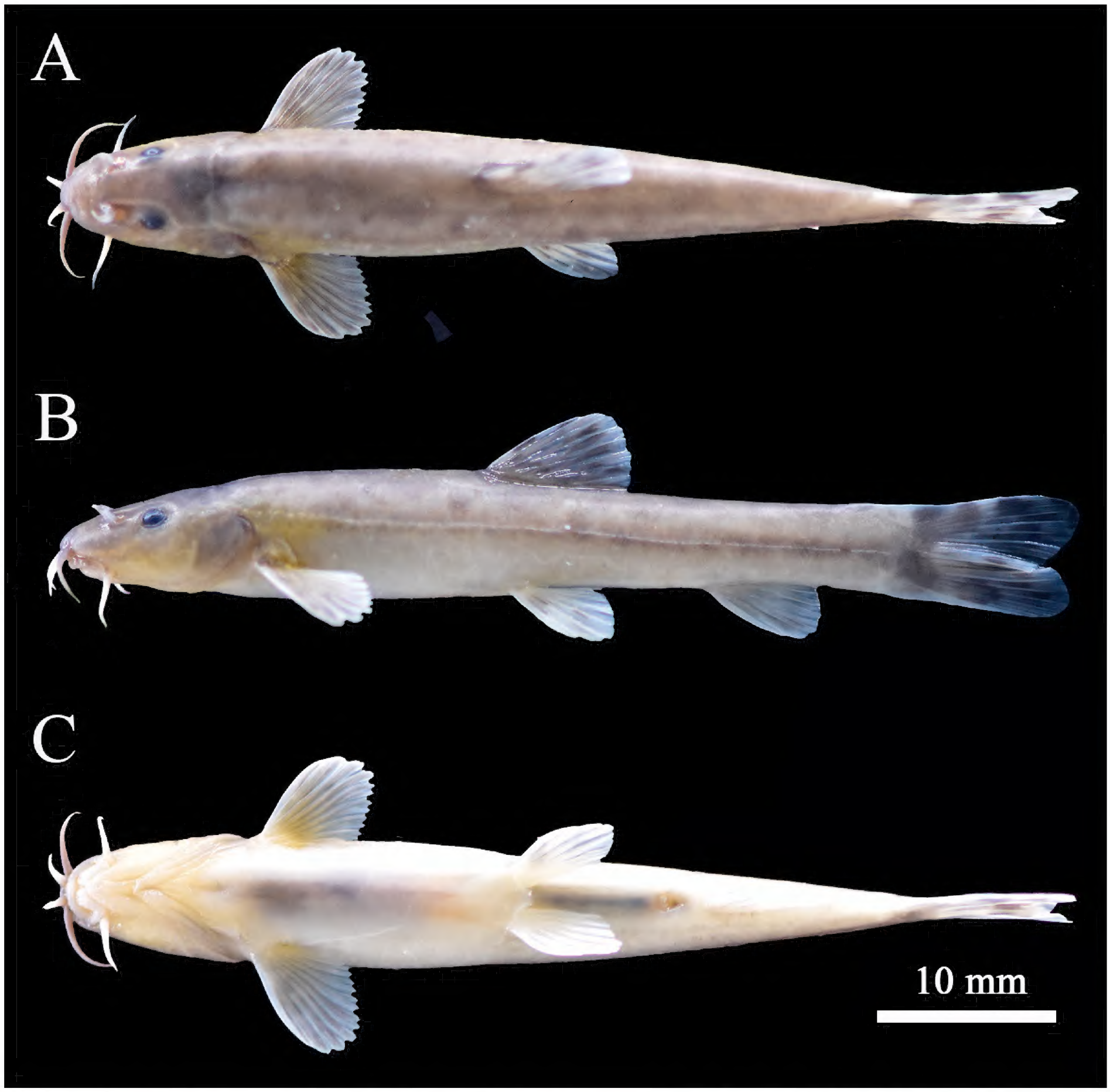

Figure 4. Morphological characteristics of *Triplophysa xingyiensis* sp. nov. in 10% formalin, GZNUSLS202507025, 70.8 mm SL. **A.** Dorsal view; **B.** Lateral view; **C.** Ventral view.

caudal fin being shallowly emarginate). *T. xingyiensis* sp. nov. can be distinguished from *T. nanpanjiangensis* by the following characteristics: the dorsal fin (ii, 7 vs. iii, 7–8), 2 unbranched pectoral fin rays (vs. 1), the pelvic fin (ii, 5 vs. i, 6), 1 unbranched anal fin ray (vs. 3). It can be distinguished from *T. tianxingensis* by dorsal fin (ii, 7 vs. iii, 8), 2 unbranched pectoral fin rays (vs. 1), 2 unbranched pelvic fin rays (vs. 1) and 1 unbranched anal fin ray (vs. 1 unbranched anal fin ray). Finally, *T. xingyiensis* sp. nov. differs from *T. xiangshuingensis* by the dorsal fin (ii, 7 vs. iii, 6), 2 unbranched pectoral fin rays (vs. 1), the pelvic fin (ii, 5 vs. i, 6), 1 unbranched anal fin ray (vs. 2) and 16 caudal fin rays (vs. 14).

Amongst the above species of *Triplophysa* in the cave plateau loach group, *T. xingyiensis* sp. nov. is most morphologically similar to *T. anlongensis*, *T. rongduensis* and *T. zhenfengensis*. Therefore, the differences between *T. xingyiensis* sp. nov. and these three species can be distinguished by further morphological characteristics.

Triplophysa xingyiensis sp. nov. can be distinguished from *T. zhenfengensis* by the caudal fin having equally large upper and lower lobes (vs. the upper lobe being larger than the lower one), anterior nostril tubular, with the end elongated into short barbels (vs. normal morphology of anterior nostril), 2 unbranched dorsal fin rays (vs. 3), 2 unbranched pectoral fin rays (vs. 1), 2 unbranched pelvic fin rays (vs. 1), 1 unbranched anal fin ray (vs. 3) and 16 caudal fin rays (vs. 14–15).

Triplophysa xingyiensis sp. nov. can be distinguished from *T. rongduensis* by having a clear cephalic canals line system (vs. unclear), dorsal fin rays (ii, 7 vs. iv, 9), pectoral fin rays (ii, 7–9 vs. i, 10), 1 unbranched anal fin ray

Figure 5. Habitat of *Triplophysa xingyiensis* sp. nov.

(vs. 3), pectoral fin tip not reaching the origin of pelvic fin (vs. tip reaching the origin of pelvic fin), the ratio of pectoral fin length to standard length being 15.2–19.8% (vs. 25.9–27.5%) and total vertebrae 4+36 (vs. 4+39).

Triplophysa xingyiensis sp. nov. can be distinguished from *T. anlongensis* by having a clear cephalic canals line system (vs. unclear), 2 unbranched dorsal fin rays (vs. 3), 2 unbranched pectoral fin rays (vs. 1) and 1 unbranched anal fin ray (vs. 3). The ratio of eye diameter to head length is 9.1–17.3% (vs. 6.6–8.7%) and the ratio of interocular distance to head length is 29.3–37.5% (vs. 20.0–24.0%).

Molecular results

The phylogenetic tree, based on the 13 protein-coding genes of the mitochondrial genome, revealed that *Triplophysa* can be divided into two distinct clades, with epigean comprising surface–dwelling species and hypogean comprising cave–dwelling species. *T. xingyiensis* sp. nov. formed a clade with 29 other cave–dwelling species of the genus *Triplophysa* (Fig. 6). The two specimens of the new species constituted a monophyletic group and exhibited consistent morphological characteristics. Furthermore, the interspecific genetic distances of Cyt *b* between *T. xingyiensis* sp. nov. and its closely-related species all exceeded 1.8% (Table 3). Therefore, *T. xingyiensis* sp. nov. was further identified as a new species within the genus *Triplophysa*.

Discussion

The uplift of the Tibetan Plateau during the Early Miocene triggered profound environmental changes across Asia and is considered a key geological event driving the origin and dispersal of the fish genus *Triplophysa* (Zhu 1989; Wu 1992; He et al. 2006). During the reorganisation of river systems driven by monsoon climate, some species within this genus dispersed to the karst regions of the Yunnan-Guizhou Plateau and gradually adapted to cave and subterranean river environments (He et al. 2006; Yan 2017). Based on morphological characteristics and mitochondrial genomic data, this study describes and confirms a new cave-dwelling species — *Triplophysa xingyiensis* sp. nov. With this addition, the number of described cave-dwelling *Triplophysa* species has increased to 45 and the number of recorded cave-dwelling *Triplophysa* species within Guizhou Province has reached 16. This discovery further enriches the species diversity of the genus and

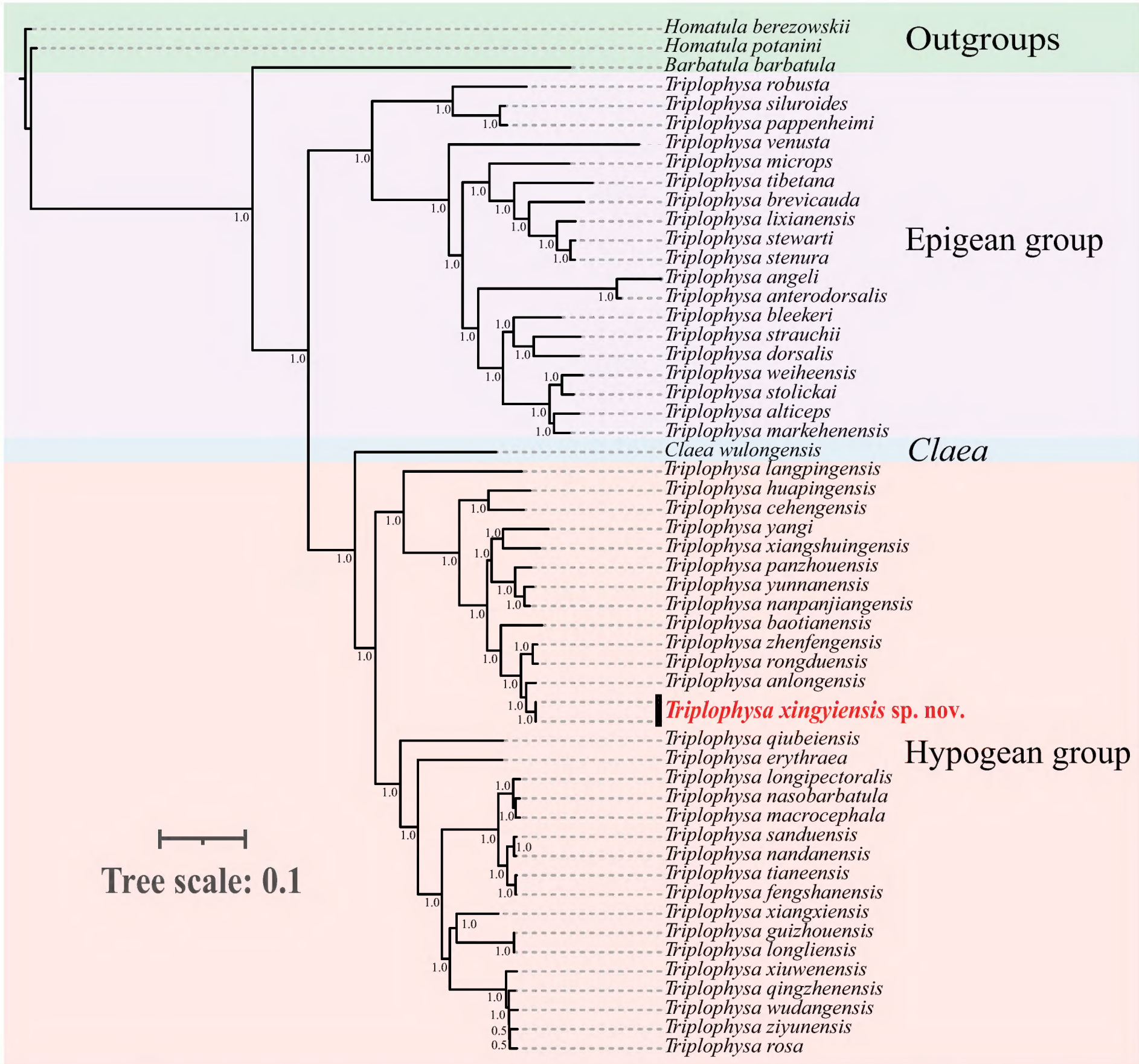

Figure 6. Bayesian Inference (BI) tree derived from 13 protein-coding genes of mitochondrial genome of fifty species of *Triplophysa*, one species of *Claea* and three outgroup species.

provides important new material for exploring the formation and adaptive evolution of subterranean biodiversity in karst regions.

Phylogenetic reconstruction, based on the 13 protein-coding genes of the mitochondrial genome, indicates that species within the genus *Triplophysa* can be divided into two major evolutionary lineages: cave-dwelling species (hypogean) and surface-dwelling species (epigean) (Fig. 6). *Triplophysa xingyiensis* sp. nov. belongs to the hypogean group, together with its closely-related species *T. anlongensis*, *T. rongduensis* and *T. zhenfengensis*, forming a well-supported monophyletic clade (Fig. 6). Ecologically, it is restricted to cave entrances or subterranean habitats and cannot complete its life cycle independently in non-cave environments (Zhao and Zhang 2006). It exhibits intermediate morphological traits: the eye diameter ranges from 9.1% to 17.3% of head length, reduced relative to surface-dwelling species, but larger than in highly degenerate, typical cave-adapted species, likely reflecting adaptation to low-light conditions near cave entrances (Xiao et al. 2025). Furthermore, the genetic distances between *T. xingyiensis* sp. nov. and its close relatives *T. anlongensis*, *T. rongduensis* and *T. zhenfengensis* are 1.8%, 2.2% and 2.4%, respectively, indicating that this species may represent an early stage of divergence (Liu et al. 2022). Cave species, due to their living in relatively enclosed environments, face unique natural selection and evolutionary pressures, which may lead to the rapid formation of new species (Lan et al. 2024).

Cave-dwelling *Triplophysa* in China exhibit pronounced spatial aggregation, with diversity concentrated in the Yunnan–Guizhou Plateau and marked gaps elsewhere (Luo et al. 2023). The increasing discoveries of these endemic and rare species in this region have raised concerns about their conservation (Luo et al.

Table 3. Kimura-2-Parameter genetic distance (in %) amongst 36 cave species of *Triplophysa*, based on the Cyt *b* gene sequences.

ID	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*T. xingyiensis* **sp. nov.**																		
2	*T. anlongensis*	1.8																	
3	*T. baishuijiangensis*	16.7	17.6																
4	*T. baotianensis*	6.2	6.6	17.0															
5	*T. cehengensis*	9.5	10.4	16.6	11.9														
6	*T. erythraea*	15.1	16.3	14.6	15.8	17.1													
7	*T. fengshanensis*	18.5	19.0	15.1	19.3	19.0	13.1												
8	*T. guizhouensis*	14.7	16.0	14.5	16.4	15.1	11.5	11.2											
9	*T. huapingensis*	10.2	10.3	16.7	11.1	5.9	17.0	18.9	16.6										
10	*T. langpingensis*	15.2	16.0	17.5	15.9	15.8	18.3	19.2	17.2	16.6									
11	*T. longipectoralis*	17.6	18.2	14.5	19.3	18.5	13.6	5.5	10.9	17.8	18.7								
12	*T. longliensis*	14.7	16.0	14.5	16.4	15.1	11.5	11.2	0.0	16.6	17.2	10.9							
13	*T. macrocephala*	16.9	17.2	14.4	18.4	17.4	13.3	5.6	10.3	17.1	18.6	1.3	10.3						
14	*T. nandanensis*	17.7	18.4	14.7	19.0	18.4	13.8	1.9	10.8	18.5	19.5	4.9	10.8	5.4					
15	*T. nanpanjiangensis*	7.6	7.8	17.2	9.0	11.5	16.3	18.4	16.8	11.2	17.2	18.1	16.8	17.5	17.9				
16	*T. nasobarbatula*	16.8	17.4	13.8	18.3	17.5	13.4	5.8	10.5	17.2	17.8	1.4	10.5	0.8	5.7	17.4			
17	*T. panzhouensis*	6.9	6.7	16.6	8.0	10.8	15.2	18.1	16.0	10.8	16.8	17.9	16.0	17.1	17.8	3.1	17.2		
18	*T. qingzhenensis*	16.6	17.3	14.1	17.4	16.1	12.5	11.0	9.6	16.8	16.6	10.9	9.6	10.6	11.2	16.9	10.5	17.3	
19	*T. qini*	16.2	16.8	13.5	18.0	16.1	11.9	11.2	5.5	17.7	18.4	10.7	5.5	10.1	11.5	17.2	10.5	16.5	9.9
20	*T. qiubeiensis*	15.1	15.9	14.8	16.1	16.6	15.7	16.5	14.6	16.5	16.7	16.5	14.6	16.5	16.3	16.9	16.0	16.7	15.4
21	*T. rongduensis*	2.2	3.3	17.2	7.0	10.0	16.4	19.4	15.3	9.8	15.8	17.8	15.3	17.5	18.3	9.0	17.2	8.4	17.4
22	*T. rosa*	16.8	17.8	13.7	17.4	16.6	12.8	11.7	9.7	17.6	16.7	11.3	9.7	10.9	11.8	17.7	10.6	17.9	1.4
23	*T. sanduensis*	17.8	18.5	14.8	19.1	18.5	13.9	2.0	10.9	18.7	19.4	5.0	10.9	5.5	0.1	18.0	5.8	17.9	11.2
24	*T. tianeensis*	18.3	18.8	14.9	19.1	18.9	12.9	0.2	11.1	18.8	19.2	5.3	11.1	5.4	1.9	18.2	5.7	17.9	10.9
25	*T. tianxingensis*	17.7	17.6	14.7	17.5	18.8	15.4	17.6	15.8	17.9	16.7	18.1	15.8	18.3	17.5	18.1	17.9	17.5	15.2
26	*T. wenshanensis*	18.7	18.5	15.4	18.3	20.3	16.4	17.8	16.4	19.2	16.9	18.1	16.4	18.4	17.6	18.9	17.8	18.6	14.7
27	*T. wudangensis*	16.7	17.6	13.8	17.0	16.3	12.5	11.6	9.9	16.9	16.6	11.3	9.9	11.0	11.7	17.5	10.7	17.8	1.7
28	*T. xiangshuingensis*	6.8	7.6	17.7	9.6	10.5	17.1	18.9	16.3	10.6	16.0	18.8	16.3	17.9	18.5	7.7	17.8	6.8	18.4
29	*T. xiangxiensis*	15.3	15.9	13.6	16.9	16.3	12.5	9.8	8.6	17.5	16.9	9.3	8.6	9.2	10.5	15.8	9.0	15.6	9.4
30	*T. xiuwenensis*	16.4	17.4	14.6	17.2	16.4	12.4	11.7	9.9	17.5	16.7	11.4	9.9	11.3	11.3	17.3	10.9	17.3	2.0
31	*T. xuanweiensiss*	15.4	16.6	9.9	16.3	16.5	13.3	13.7	12.9	16.5	16.1	13.2	12.9	12.9	13.1	16.0	12.8	15.8	12.9
32	*T. yaluwang*	14.6	15.9	14.0	15.9	15.5	11.3	10.8	1.5	16.2	17.2	10.6	1.5	10.1	10.6	16.3	10.2	15.2	9.3
33	*T. yangi*	7.7	7.9	18.6	8.6	11.8	18.3	20.6	17.1	12.1	16.6	19.5	17.1	18.4	20.2	8.5	18.8	8.4	18.7
34	*T. yunnanensis*	7.6	8.0	17.3	8.9	11.6	16.6	18.1	16.8	11.3	17.2	18.3	16.8	17.7	17.9	1.2	17.5	3.2	16.5
35	*T. zhenfengensis*	2.4	3.3	17.7	7.4	10.1	16.4	19.6	15.9	10.0	16.0	18.3	15.9	18.0	18.8	9.0	17.7	8.6	17.6
36	*T. ziyunensis*	16.6	17.5	13.9	16.9	16.1	12.1	11.3	10.1	16.9	16.0	11.0	10.1	10.7	11.6	17.4	10.4	17.6	1.6

Table 3. Continued..

ID	Species	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	*T. xingyiensis* **sp. nov.**																	
2	*T. anlongensis*																	
3	*T. baishuijiangensis*																	
4	*T. baotianensis*																	
5	*T. cehengensis*																	
6	*T. erythraea*																	
7	*T. fengshanensis*																	
8	*T. guizhouensis*																	
9	*T. huapingensis*																	
10	*T. langpingensis*																	
11	*T. longipectoralis*																	
12	*T. longliensis*																	
13	*T. macrocephala*																	
14	*T. nandanensis*																	
15	*T. nanpanjiangensis*																	
16	*T. nasobarbatula*																	
17	*T. panzhouensis*																	
18	*T. qingzhenensis*																	
19	*T. qini*																	
20	*T. qiubeiensis*	15.8																
21	*T. rongduensis*	16.9	15.4															
22	*T. rosa*	10.3	15.6	17.6														
23	*T. sanduensis*	11.6	16.4	18.4	11.8													
24	*T. tianeensis*	11.2	16.3	19.1	11.7	2.0												
25	*T. tianxingensis*	15.3	12.4	17.6	16.0	17.6	17.5											
26	*T. wenshanensis*	16.3	12.9	18.8	15.1	17.7	17.7	3.3										
27	*T. wudangensis*	10.5	16.0	17.5	1.5	11.7	11.5	15.7	15.1									
28	*T. xiangshuingensis*	17.5	15.5	8.0	18.7	18.6	18.7	17.3	19.0	18.6								
29	*T. xiangxiensis*	6.4	15.8	16.0	9.5	10.6	9.7	14.5	15.7	9.8	17.0							
30	*T. xiuwenensis*	10.3	15.6	17.2	2.2	11.3	11.7	14.5	14.1	2.3	18.4	8.8						
31	*T. xuanweiensiss*	12.8	14.1	16.8	13.0	13.2	13.5	15.0	14.1	12.5	17.1	12.8	13.4					
32	*T. yaluwang*	5.6	14.4	15.4	9.6	10.7	10.8	14.9	15.8	9.6	16.3	8.6	9.7	12.5				
33	*T. yangi*	18.1	16.7	7.7	19.2	20.3	20.4	18.6	19.5	19.2	7.2	17.5	19.0	18.3	17.2			
34	*T. yunnanensis*	17.1	16.6	9.2	17.0	18.0	17.9	18.2	18.7	17.0	7.7	15.9	17.2	16.4	16.3	9.0		
35	*T. zhenfengensis*	17.3	15.4	0.9	17.9	18.9	19.4	18.1	19.0	17.7	8.2	16.2	17.5	16.8	16.0	8.2	9.2	
36	*T. ziyunensis*	10.4	15.7	17.4	1.4	11.5	11.2	15.6	14.9	1.4	18.7	9.7	2.2	12.2	10.0	19.2	17.0	17.6

2023; Cao et al. 2025; Shi et al. 2025; Zhu et al. 2025). These specialised habitats face ongoing threats from anthropogenic activities, such as groundwater pollution. Conservation strategies should leverage species distribution models to delineate suitable habitats and optimise protection (Elith and Leathwick 2009). Overall, this study not only describes a new species, but also provides insights into the phylogenetic patterns and biogeographical history underlying the diversification of cave-adapted *Triplophysa* in karst regions.

Author contributions

Ren-Yi Zhang designed the study and revised the manuscript. Feng-Hua Yuan carried out morphological description, molecular analysis and drafted the manuscript. Ting-Ting Zhu participated in the morphological measurement, molecular analysis and revised the manuscript. All authors have read and approved the final version of the manuscript.

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Appendix 1

Comparative material

T. anlongensis (n = 3): China: Guizhou: Anlong County: Xinglong Town: GZNUSLS202501337–GZNUSLS202501339.

T. baotianensis (n = 3): China: Guizhou: Panzhou City: Baotian Town: GZNUSLS202201086–GZNUSLS202201088.

T. cehengensis (n = 3): China: Guizhou: Ceheng County: Rongdu Town: GZNUSLS202407006–GZNUSLS202407008.

T. erythraea (n = 7): China: Hunan: Fenghuang County: Heku Town: GZNUSLS202503019–GZNUSLS202503025.

T. guizhouensis (n = 7): China: Guizhou: Huisui County: Baijin Town: GZNUSLS202502001–GZNUSLS202502003, GZNUSLS202502028–GZNUSLS202502031.

T. huapingensis (n = 4): China: Guangxi: Leye County: Chaoyang Town: GZNUSLS210702498–GZNUSLS210702501.

T. langpingensis (n = 4): China: Guangxi: Tianlin County: Langping Town: GZNUSLS202501312–GZNUSLS202501315.

T. longliensis (n = 3): China: Guizhou: Longli County: Basheng Township: GZNUSLS022072266–GZNUSLS022072268.

T. nanpanjiangensis (n = 5): China: Yunnan: Qujing City: Zhanyi District: GZNUSLS202407044–GZNUSLS202407048.

T. nasobarbatula (n = 11): China: Guizhou: Libo County: Maolan Town: GZNUSLS202001339–GZNUSLS202001349.

T. panzhouensis (n = 4): China: Guizhou: Panzhou City: Hongguo Town: GZNUSLS202304280–GZNUSLS202304283.

T. qingzhenensis (n = 11): China: Guizhou: Guiyang City: Qingzhen City: GZNUSLS202110074–GZNUSLS202110084.

T. qiubeiensis (n = 3): China: Yunnan: Qiubei County: Shuanglongying Town: GZNUSLS202501318–GZNUSLS202501320.

T. rongduensis (n = 5): China: Guizhou: Ceheng County: Rongdu Town: GZNUSLS202407001–GZNUSLS202407005.

T. rosa (n = 3): China: Chongqing City: Wulong District: Jiangkou Town: GZNUSLS202503092–GZNUSLS202503094.

T. sanduensis (n = 3): China: Guizhou: Sandu County: Zhonghe Town: GZNUSLS202201082–GZNUSLS202201084.

T. tianxingensis (n = 2): China: Yunnan: Guangnan County: Zhulin Town: GZNUSLS202309181–GZNUSLS202309182.

T. tianeensis (n = 11): China: Guangxi: Tian'e County: Bala Yao Ethnic Township: GZNUSLS202504009–GZNUSLS202504019.

T. wudangensis (n = 2): China: Guizhou: Guiyang City: Wudang District: GZNUSLS202410001–GZNUSLS202410002.

T. xiangshuingensis (n = 4): China: Yunnan: Shilin County: Lufu Town: GZNUSLS202505002–GZNUSLS202505005.

T. xiuwenensis (n = 4): China: Guizhou: Xiuwen County: Dashi Buyi Ethnic Township: GZNUSLS202306086, GZNUSLS202306088–GZNUSLS202306090.

T. yangi (n = 3): China: Yunnan: Shizong County: Wulong Township: GZNUSLS202304272–GZNUSLS202304274.

T. yunnanensis (n = 8): China: Yunnan: Yiliang County: Jiuxiang Yi and Hui Ethnic Township: GZNUSLS202304263–GZNUSLS202304265, GZNUSLS202501332–GZNUSLS202501336.

T. zhenfengensis (n = 7): China: Guizhou: Zhengfeng County: Longchang Town: GZNUSLS202201089–GZNUSLS202201091, GZNUSLS202311040–GZNUSLS202311043.

T. ziyunensis (n = 5): China: Guizhou: Zhenning County: GZNUSLS202201077–GZNUSLS202201081.

The following is comparative material from the references: *T. aluensis* (Li and Zhu 2000), *T. anshuiensis* (Wu et al. 2018a), *T. baishuijiangensis* (Shi et al. 2025), *T. fengshanensis* (Lan et al. 2013), *T. flavicorpus* (Yang et al. 2004), *T. gejiuensis* (Chu and Chen 1979), *T. longipectoralis* (Zheng et al. 2009), *T. luochengensis* (Li et al. 2017a), *T. macrocephala* (Yang et al. 2012), *T. nandanensis* (Lan et al. 1995), *T. posterodorsalus* (Ran et al. 2006), *T. qini* (Deng et al. 2022), *T. shilinensis* (Chen et al. 1992), *T. tianlinensis* (Li et al. 2017b), *T. wenshanensis* (Cao et al. 2025), *T. xiangxiensis* (Yang et al. 1986), *T. xichouensis* (Liu et al. 2017), *T. xuanweiensis* (Lu et al. 2022) and *T. yaluwang* (Lan et al. 2024).